INFECTION AND IMMUNITY, Mar. 2005, p. 1295–1303 Vol. 73, No. 3
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.3.1295–1303.2005

The StcE Protease Contributes to Intimate Adherence of Enterohemorrhagic *Escherichia coli* O157:H7 to Host Cells

Thomas E. Grys, Matthew B. Siegel, Wyndham W. Lathem,† and Rodney A. Welch*

Department of Medical Microbiology and Immunology, University of Wisconsin—Madison, Madison, Wisconsin

Received 16 July 2004/Returned for modification 22 October 2004/Accepted 8 November 2004

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a diarrheal pathogen that causes attaching and effacing (A/E) lesions on intestinal epithelial cells. Strains of the O157 serogroup carry the large virulence plasmid pO157, which encodes the *etp* type II secretion system that secretes the genetically linked zinc metalloprotease StcE. The Ler regulator controls expression of many genes involved in A/E lesion formation, as well as StcE, suggesting StcE may be important at a similar time during colonization. Our laboratory has previously demonstrated that StcE cleaves C1-esterase inhibitor, a regulator of multiple inflammation pathways. Here we report two new substrates for StcE, mucin 7 and glycoprotein 340, and that purified StcE reduces the viscosity of human saliva. We tested the hypothesis that StcE contributes to intimate adherence of EHEC to host cells by cleavage of glycoproteins from the cell surface. The fluorescent actin stain (FAS) test was used to observe the intimate adherence represented by fluorescently stained bacteria colocalized with regions of bundled actin formed on HEp-2 cells. An *E. coli* O157:H7 strain with a *stcE* gene deletion was not affected in its ability to generally adhere to HEp-2 cells, but it did score threefold lower on the FAS test than wild-type or complemented strains. Addition of exogenous recombinant StcE increased intimate adherence of the mutant to wild-type levels. Thus, StcE may help block host clearance of *E. coli* O157:H7 by destruction of some classes of glycoproteins, and it contributes to intimate adherence of *E. coli* O157:H7 to the HEp-2 cell surface.

Enterohemorrhagic *Escherichia coli* (EHEC) strains are food-borne human pathogens with an infectious dose of approximately 100 CFU (56). These bacteria colonize the colon, where they cause painful diarrhea that frequently becomes bloody. The disease may progress to hemolytic uremic syndrome and death.

During the course of an infection, EHEC must evade or overcome several of the body's defense mechanisms. In the oral cavity EHEC encounters saliva, one of the host's first defenses. Saliva provides a physical barrier to protect the oral epithelium and contains mucins, soluble immunoglobulin A, and proteins that can aggregate pathogens like EHEC. Phagocytic cells such as macrophages and neutrophils can then interact with and engulf these bacteria-protein aggregates as well as individual bacteria (35, 63). EHEC cells that make it through the oral cavity to the stomach are confronted with extremely low pH, which is deadly to many bacteria. EHEC, however, is remarkable for its ability to tolerate this acidity with little loss of viability (3, 6, 12). After the stomach, EHEC enters the small and large intestines, where pH levels progressively rise and provide an amenable environment for growth. Typically, EHEC colonizes the colon, where there is intense competition for space and resources from 10^{13} bacteria representing over 400 species (7). There, EHEC must not only survive but also penetrate the mucus layer of the intestinal epithelium to adhere intimately to host cells by forming attaching and effacing (A/E) lesions, thus establishing an infection.

The remarkably low infectious dose suggests the bacterium possesses traits to overcome multiple host innate defenses. Some of the mechanisms that EHEC uses to establish an infection, such as acid tolerance and A/E formation, are being actively investigated and understood in increasing detail. Other mechanisms, such as the ability to evade oral defenses, traverse a thick mucus barrier, and successfully grow in a highly competitive microbial environment, are not known.

The A/E lesion results in intimate adherence of EHEC to the host cell membrane and a rearrangement of host cell actin microvilli (reviewed in references 29 and 56). The bacterium uses a type III secretion system (T3SS) to inject a bacterium-encoded protein, Tir, into the host cell, where it is displayed on the host cell surface (40). Tir is the primary bacterial receptor and is bound by the intimin adhesin on the bacterial surface (36, 37). Tir and other T3SS proteins rearrange host cell actin, leading to effacement of the microvilli. The actin is bundled and pushes the bacterium up above the host cell surface, creating a pedestal structure. The locus of enterocyte effacement (LEE) element encodes intimin, Tir, the T3SS, and other proteins necessary for pedestal formation (49).

E. coli O157:H7 carries a 92-kb virulence plasmid, pO157, which encodes a number of potential virulence factors (13). One of these genes encodes StcE, a zinc metalloprotease that our laboratory showed is secreted by the closely linked *etp* type II secretion system on pO157. Expression of the *stcE* gene is

* Corresponding author. Mailing address: Department of Medical Microbiology and Immunology, University of Wisconsin—Madison, 1300 University Ave., Room 481 MSC, Madison, WI 53706. Phone: (608) 263-2700. Fax: (608) 262-8418. E-mail: rawelch@wisc.edu.

† Present address: Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110.

up-regulated by the global regulator Ler, which is encoded on the chromosomal LEE element and regulates four of the five LEE operons (45, 51).

Our laboratory has demonstrated that StcE is not a general protease but has only one identified substrate, C1-esterase inhibitor (C1-INH). C1-INH is a serine protease inhibitor (serpin) and regulator of the complement system and other mechanisms of inflammation (14). Interestingly, StcE-cleaved C1-INH retains functional serpin activity to inhibit the classical complement cascade (43). Furthermore, StcE binds and localizes functional C1-INH to cell membranes, effectively enhancing the ability of C1-INH to regulate complement effectors at sites of potential lytic complex formation. Thus, our model of the StcE–C1-INH interaction is that StcE potentiates C1-INH activity to reduce inflammation and complement-mediated lysis at the site of infection (43). This activity would be especially important when Shiga-like toxins and other EHEC effectors compromise the intestinal barrier, resulting in the entry of blood and complement proteins to the intestinal lumen.

Here we identify two additional substrates for StcE activity, glycoprotein 340 (gp340) and mucin 7 (MUC7), and report that StcE reduces the viscosity of human saliva. The substrates are heavily glycosylated proteins with defensive roles found in saliva and other tissues (10, 35, 53). The mucinase activity of StcE and the fact that its expression is coregulated with the pedestal-forming proteins of the LEE element led us to the hypothesis that StcE contributes to intimate adherence of *E. coli* O157:H7 to host cells by degrading the protective layer of mucins and glycoproteins on the host cells. Herein we provide evidence to support this hypothesis and a role for StcE in establishing *E. coli* O157:H7 infection by helping the bacterium evade the mucosal defenses of the host. A model that reconciles the mucinase activity of StcE with its C1-INH cleaving activity is presented.

MATERIALS AND METHODS

Strains and media. For all cloning steps, bacteria were grown using Luria-Bertani broth or plates (Fisher Scientific, Pittsburgh, Pa.). Strains used were EDL933, a model strain for EHEC pathogenesis isolated from ground beef (61); WAM2871, *E. coli* carrying the helper plasmid pUX-BF13 encoding Tn7 transposase genes *tnsABCDE* (21); and WAM1301, the *E. coli* S17λpir host strain.

Strain construction. An EDL933 *stcE* deletion mutant was constructed by the linear recombination (λ Red) method of Datsenko and Wanner (20). Briefly, the oligonucleotides 5′ 707 (5′-ATG AAA TTA AAG TAT CTG TCA TGT ACG ATC CTT GCC CCT TGT GTA GGC TGG AGC TGC TTC-3′) and 3′ 708 (5′-TAA TTT ATA TAC AAC CCT CAT TGA CCT AGG TTT ACT GAA GCA TAT GAA TAT CCT CCT TAG-3′) were used to amplify by PCR the chloramphenicol resistance cassette from the nonpolar (in frame, with added ribosome binding site) plasmid template pKD3. The resulting product was then transformed by electroporation into WAM2806 (EDL933 carrying pKD46, grown at 30°C in the presence of 10 mM arabinose). Integrates cured of pKD46 and lacking the *stcE* coding sequence were selected by growth on LB agar containing chloramphenicol (20 μg/ml) at 42°C, and mutations were confirmed by PCR. More than 95% of the coding sequence of *stcE* was deleted, leaving behind the 5′ and 3′ ends of the gene encoded by the oligonucleotides. The strain was designated WAM2815.

A strain having the *stcE* gene complemented was created using a Tn7 transposase system (21). The *stcE* gene was amplified from purified pO157 template by PCR using primers 5′ 1135 (5′-AAG GGC CCC TCT GAG GTG TCT GTT AAA CCC GTG G-3′) and 3′ 1136 (5′-AAA AA TGG CCA CGA AGT GGC CGC ACC GTC TCA GG-3′). The gene was inserted into the ApaI-MscI sites of pEVS107 and transformed by electroporation into the donor strain WAM1301, creating a strain called WAM2980. The strains WAM2980 and WAM2871 were conjugated with WAM2815. This resulted in strain WAM2997, which is the WAM2815 Δ*stcE*::cm strain that carries a single copy of *stcE* on the chromosome at the *glmS* Tn7*att* site and has restored StcE expression (data not shown).

Production of rStcE′. Recombinant StcE protein was created using the IMPACT protein expression system from New England Biolabs (NEB; Beverly, Mass.). Briefly, nucleotides 23121 to 25712 of the *E. coli* O157:H7 plasmid pO157 (accession number AF074613) were amplified by PCR using Deep Vent polymerase (NEB) and purified pO157 plasmid DNA as a template. This sequence encodes the secreted form of StcE that lacks the signal peptide, called StcE′ (accession number AY714880). The gene was then ligated into pTYB1 (NEB) at the NheI and SapI restriction sites of the multiple cloning site, creating pTEG11. This plasmid contains a fusion gene of *stcE* with sequences encoding a chitin binding domain and an intein protease. The plasmid was transformed into the *E. coli* expression strain ER2566 (NEB). The chitin binding domain of the expressed protein allowed affinity purification on a chitin column, while the intein protease allowed the target protein to be released from the two fusion domains. The recombinant StcE′ (rStcE′) protein has three extra N-terminal residues (Met-Ala-Ser) but is otherwise identical to the protein sequence secreted by strains carrying pO157.

Identification of proteins cleaved by StcE. Whole human saliva was collected from an investigator, and samples (20 μl) were treated with rStcE′ (1 μg) or untreated for 2 h at room temperature. Proteins were boiled in sample buffer and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 8% acrylamide) and stained with Coomassie R-250. Two proteins of interest (approximately 300 and 150 kDa) were excised from the gel, digested with trypsin, and analyzed by matrix-assisted laser desorption ionization–time-of-flight spectrometry at the UW Biotechnology Center Proteomics/Mass Spectrometry Facility. Peptide masses were compared to those in the MASCOT database (www.MatrixScience.com) for identification. The results for the 150-kDa protein did not suggest a protein identification, though review of the literature for saliva proteins of similar size did suggest probable identities. The identities were confirmed by immunoblotting with a monoclonal antibody against gp340 (mouse anti-human; 1:10,000; MBL, Woburn, Mass.) and polyclonal MUC7 antiserum (mouse anti-human; 1:1000; kind gift of J. G. M. Bolscher). Other substrates tested included lactoferrin, porcine gastric mucin, bovine fetuin, bovine submaxillary mucin, and ovalbumin, all purchased from Sigma.

Viscosity determinations. Whole human saliva was collected from an investigator, cleared by centrifuging for 30 min at 12,000 × *g* at 4°C, and split into 10-ml samples. The viscometer and samples were equilibrated at 37°C prior to treatment and measurement. The viscosity of the samples was measured before and after a 3-h treatment with rStcE′ (70 μg) or buffer (20 mM Tris, pH 7.5). Both the treatment and measurements took place at 37°C. Relative viscosity was assessed by measuring elution time in a Cannon-Fenske routine viscometer (range, 0.5 to 4 cP; Cannon Instrument Company, State College, Pa.). The elution time for water was considered 0% viscosity, while buffer-treated saliva was considered 100%. Two to four measurements were taken per time point, and the experiment was repeated three times.

Tissue culture. HEp-2 cells were maintained in Eagle's modified minimum essential medium (Mediatech, Herndon, Va.) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, Ga.), 10 mM sodium pyruvate, penicillin, streptomycin, and amphotericin B. After achieving confluence, cells were passaged by lifting with 0.25% trypsin–EDTA (Mediatech) and diluted to 1:5 or 1:10.

Biotinylation experiments. Proteins on HEp-2 cell surfaces were biotinylated on carboxyl and aldehyde groups by using biotin hydrazide with 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) according to the manufacturer's protocol (Pierce, Rockford, Ill.). Cells were then incubated in phosphate-buffered saline (PBS) or PBS plus rStcE′ (5 μg/ml) at 37°C. After 2 h the samples were separated into supernatant and two detergent-soluble cell fractions (2% saponin–PBS and 0.5% digitonin–saponin–PBS). The biotinylated proteins were captured using NeutrAvidin immobilized on beads (Pierce). Beads from each fraction were then washed, boiled in sample buffer, and separated on both an 8% acrylamide gel and a 1% agarose gel. Proteins were blotted to nitrocellulose and probed with streptavidin-horseradish peroxidase (Kirkegaard & Perry Laboratories, Gaithersburg, Md.). The blot was then visualized by exposure to Hyperfilm (Amersham Biosciences Corp., Piscataway, N.J.).

Fluorescent actin staining (FAS). An adaptation of methods described by Knutton et al. (41) and Donnenberg and Nataro (23) was employed. HEp-2 cells were grown in eight-well slides (Nalge Nunc International, Naperville, Ill.) for 24 to 48 h (50 to 80% confluent). Cells were washed three times in PBS to remove any residual antibiotics on the medium. Bacterial strains were inoculated from a single colony on an LB plate into Lennox broth (46) and grown to stationary phase (12 to 18 h) at 37°C without shaking. All strains grew to similar densities

(CFU per milliliter). The medium used for infection was Dulbecco's modified minimum essential medium supplemented as above but without antibiotics. The overnight bacterial culture was diluted 1:25 in this medium for an average inoculation of 8×10^6 CFU/ml. The medium-bacteria mixture (0.25 ml) was placed on the cells and incubated at 37°C for a total of 6 to 7 h. The medium was removed at midpoint of incubation and replaced with fresh medium with no additional bacteria.

After infection, cells were washed thoroughly (five to six times) with PBS and fixed with paraformaldehyde (3% for 10 min). Cells were washed with PBS (three times for 3 min) after fixation. A 15-min blocking step with antibody dilution solution (2% bovine serum albumin, 0.1% Triton X-100 in PBS) was used to inhibit nonspecific staining. Bacteria were treated with a monoclonal antibody (1:200; 30 min) specific for the O157 O-antigen (U.S. Biologicals, Swampscott, Mass.). After washing in PBS, cells were stained with the secondary antibody solution containing goat anti-rabbit antibody conjugated to Alexa 488 (1:1,000; Molecular Probes) and the actin-staining fungal toxin phalloidin, conjugated to Alexa 594 (1:400, Molecular Probes).

Samples were analyzed using a Zeiss fluorescence microscope and a 40× plan apochromat numerical aperture 1.3 objective (Carl Zeiss MicroImaging Inc., Thornwood, N.Y.). Images were acquired with an Axiocam monochrome charge-coupled device camera (Zeiss) and OpenLab software (Improvision, Lexington, Mass.). Samples were blindly coded to prevent potential bias. Random fields from sample wells were selected out of focus on the phalloidin channel such that no actin bundles were discernible that might bias field selection. Approximately 10 fields of each sample were imaged, and the number of foci that had bundled actin were counted. Foci were defined as either a single bacterium or a cluster of bacteria separated from other foci by more than the length of a bacterium. The experiment was repeated five times with similar results.

Statistical methods. The means of the viscosity determinations ($n = 4$ measurements per sample) were compared using the Student t test. FAS data from the five experiments were combined and analyzed using a one-way analysis of variance test to determine if all the means were equal. A Dunnett's posttest was then used to compare the means of different samples to that of the control (EDL933) and to determine P values. Numbers of fields observed for each sample were as follows: EDL933 ($n = 54$), EDL933 plus rStcE′ ($n = 34$), WAM2815 ($n = 68$), WAM2815 plus rStcE′ ($n = 58$), WAM2997 ($n = 33$).

Nucleotide sequence accession numbers. The sequence for the secreted form of StcE has been deposited in the GenBank database under accession number AY714880. The pO157 plasmid is available in GenBank under accession number AF074613.

RESULTS

StcE cleaves gp340 and MUC7. The only previously known substrate for the StcE protease was C1-INH (45). We hypothesized that saliva would provide a potential source of substrates, as it is the first host tissue that EHEC encounters. The rStcE′ protease was incubated with whole human saliva, and the proteins were separated by SDS-PAGE. The results (Fig. 1A) demonstrated that the migration of two Coomassie-stained, large-molecular-weight protein bands changed as a result of rStcE′ treatment. Treatment with a proteolytically inactive rStcE′ mutant (45) did not cause this migration change (data not shown). The larger protein (~300 kDa) was identified by mass spectrometry as gp340, a common salivary protein that is encoded by an alternately spliced variant of the gene *DMBT1* (deleted in malignant brain tumors 1). The smaller protein did not yield useful mass spectrometry data but, based on the literature and its size (72), was surmised to be MUC7. Both proteins were positively identified as substrates in immunoblot assays with antiserum to MUC7 (Fig. 1B) and antibody to gp340 (Fig. 1C).

StcE reduces the viscosity of saliva. Heavily glycosylated proteins such as gp340, MUC7, and other mucins and glycoproteins are largely responsible for the viscosity of mucous material such as saliva. We hypothesized that StcE cleavage of these proteins may result in reduced viscosity of saliva. This hypothesis was tested using a Cannon-Fenske viscometer, where flow rate through a capillary tube measures relative viscosity. Addition of rStcE′ caused a 66% reduction in viscosity of saliva compared to that of water (data not shown). This difference was statistically significant ($P < 0.0001$), and the experiment was repeated three times with similar results.

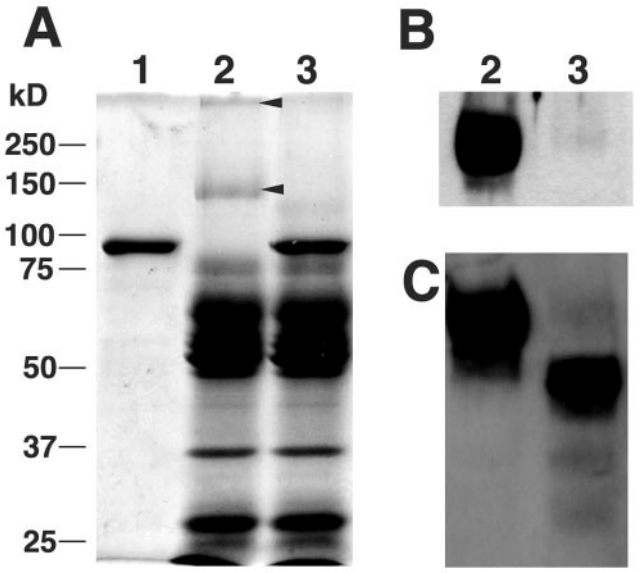

FIG. 1. Whole human saliva proteins were untreated or StcE treated and separated by SDS-PAGE. (A) Coomassie-stained gel. Two large-molecular-weight protein bands that changed as a result of rStcE′ treatment are indicated by arrowheads. (B and C) Immunoblots probed with antiserum against MUC7 (B) and antibody against gp340 (C). Lanes in all three panels: 1, rStcE′; 2, untreated saliva; 3, rStcE′-treated saliva.

StcE does not cleave all glycosylated proteins tested. StcE activity against lactoferrin, ovalbumin, bovine submaxillary mucin, bovine fetuin, and porcine gastric mucin was tested (data not shown). The results demonstrated that StcE did not cleave these proteins.

StcE alters HEp-2 cell surface proteins. The finding that StcE could cleave two known mucin-like proteins and reduce viscosity of a glycoprotein-containing material led to the hypothesis that StcE may cleave mucin-like proteins from the surfaces of host cells. To test this hypothesis, we biotinylated HEp-2 cells with biotin-hydrazide and EDC. This treatment labels surface proteins via cross-linking to carboxyl and aldehyde groups. Supernatants (S1) of untreated or StcE-treated cells contained secreted proteins or proteins cleaved from cells by StcE. The detergent-soluble fractions (S2 and S3) contained cell-associated proteins. Cell surface-associated proteins were biotinylated and were captured using NeutrAvidin beads. Analysis of samples separated by SDS-PAGE and blotted showed that supernatants from rStcE′-treated cells contained additional bands not present in the untreated sample, and the cell-associated fractions contained two bands that were reduced or missing in the rStcE′-treated samples (Fig. 2A). The biotinylated rStcE′-treated samples were separated on an agarose gel in order to observe larger-molecular-weight proteins, such as mucins. The blot of the agarose gel showed similar results (Fig. 2B) to those seen in Fig. 2A, where bands in the

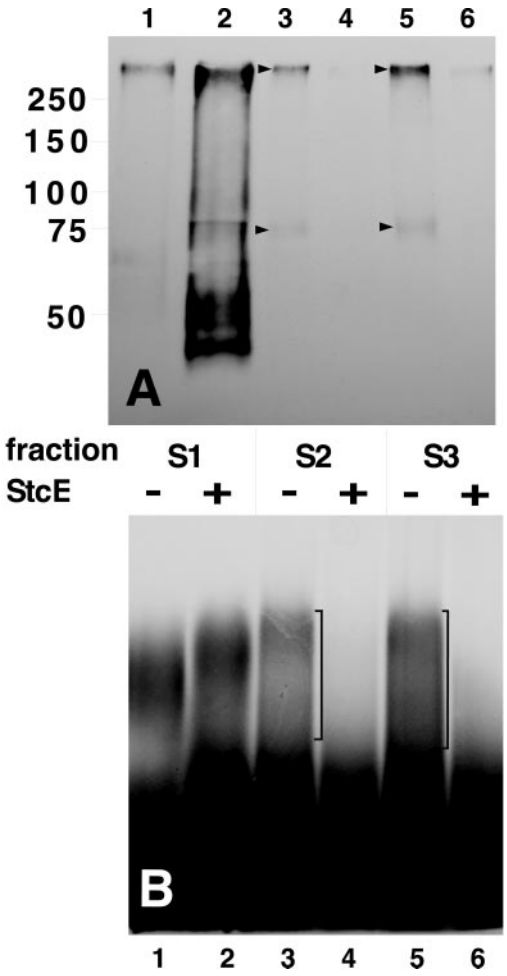

FIG. 2. Blots of biotinylated proteins of buffer- or StcE-treated HEp-2 cells. HEp-2 cell surface proteins were labeled with biotin, treated with buffer or rStcE′, and then harvested in three fractions: S1, proteins collected in supernatant; S2, cellular proteins soluble in 2% saponin; S3, cellular proteins soluble in 0.5% digitonin and 2% saponin. Fractioned proteins were separated by SDS-PAGE (A) or by SDS-agarose gel (B) and blotted to nitrocellulose. Streptavidin-horseradish peroxidase was used to detect biotinylated proteins. Lanes 1 and 2, S1 proteins from buffer- and StcE-treated cells; lanes 3 and 4, S2 proteins from buffer- and StcE-treated cells; lanes 5 and 6, S3 proteins from buffer- and StcE-treated cells. Arrowheads in panel A indicate bands in cell-associated fractions that were reduced or missing in the rStcE′-treated samples. Brackets in panel B indicate S2 and S3 fractions that disappeared after rStcE′ treatment.

S2 and S3 fractions disappeared after rStcE′ treatment. Attempts to identify the proteins in these bands by mass spectrometry and immunoblotting with antiserum to MUC7 or antibody to gp340 were unsuccessful (data not shown).

StcE contributes to intimate adherence. The demonstration that StcE cleaves large proteins from host cell surfaces suggested that StcE might play a role in adherence of *E. coli* O157:H7 to host cells. We tested this by measuring viable CFU adhering to HEp-2 cells after 3 or 6 h of infection. The results indicated that general adherence of wild-type EDL933 or an isogenic *stcE* deletion mutant (WAM2815) was the same (data not shown). We then tested if StcE contributed to intimate adherence of the bacterium to host cells. We used the FAS test (23, 41) to measure the number of bacterial foci that formed actin bundles on HEp-2 cells, the prototypic cell line for such experiments. We found that the *stcE* deletion strain WAM2815 scored threefold lower on the FAS test than the isogenic wild-type strain or the *stcE*-complemented strain, WAM2997, after a 6-h infection (Fig. 3). In addition to genetic complementation, addition of rStcE′ protein at the beginning of the infection could abolish the *stcE* deficiency of the WAM2815 strain. The FAS experiments also concurred with previous experiments suggesting that StcE did not contribute to general adherence. The important finding was the difference in intimate adherence, a step thought to be important in establishing colonization.

DISCUSSION

Our laboratory previously showed that *E. coli* O157:H7 strain EDL933 secretes StcE, an extracellular zinc metalloprotease. In our initial studies (45), StcE did not behave as a general protease active against many common protein substrates but appeared to only cleave the complement regulator, C1-INH. In further studies, proteins from cell lysates (HEp-2, Caco-2, and LS174T cells) treated with StcE that were separated by SDS-PAGE and stained with Coomassie blue showed no bands that shifted or decreased in intensity. In this study, however, we identified two salivary glycoproteins, gp340 and MUC7, as substrates for StcE proteolysis. We additionally showed that purified rStcE′ reduces the viscosity of human saliva by 66%, probably the result of this StcE mucinase activity. We propose that StcE may contribute to the low infectious dose of *E. coli* O157:H7 by preventing the formation of mucin-bacteria aggregates that could lead to bacterial elimination in mucosal environments such as the oral cavity. Furthermore, we demonstrate that StcE degrades several large cell surface proteins on HEp-2 cells. Coincident with or because of this activity, *E. coli* O157:H7 expressing StcE has an increased ability to carry out intimate adherence with HEp-2 cells as measured by FAS. Our model (Fig. 4) suggests that StcE mediates increased intimate adherence by reducing the mucous glycocalyx cell surface barrier, allowing a closer interaction between the bacterium and host cell. In the colon, StcE cleavage of mucins and glycocalyx matrix could also lead to a reduction of commensal bacteria in the immediate area, thereby enhancing the ability of *E. coli* O157:H7 to compete for space and nutrients.

We recently demonstrated that StcE interaction with C1-INH surprisingly potentiates rather than destroys C1-INH regulation of classical complement activation. StcE binds to bacterial or host cell surfaces and then localizes active C1-INH, which subsequently reduces complement-mediated lysis of both bacteria and host cells (43). Therefore, we believe the mucinase activity of StcE is important in early stages of colonization, while the StcE-mediated potentiation of C1-INH activity may be important during the bloody colitis phase of *E. coli* O157:H7 disease. At this stage it is hypothesized that Shiga-like toxins and other effectors compromise the intestinal epithelial barrier and damage the intestinal endothelium, which results in entry of blood and serum effectors into the intestinal lumen (56). As a corollary to the bifunctional model of StcE activity, we hypothesize that the kinetics and binding of

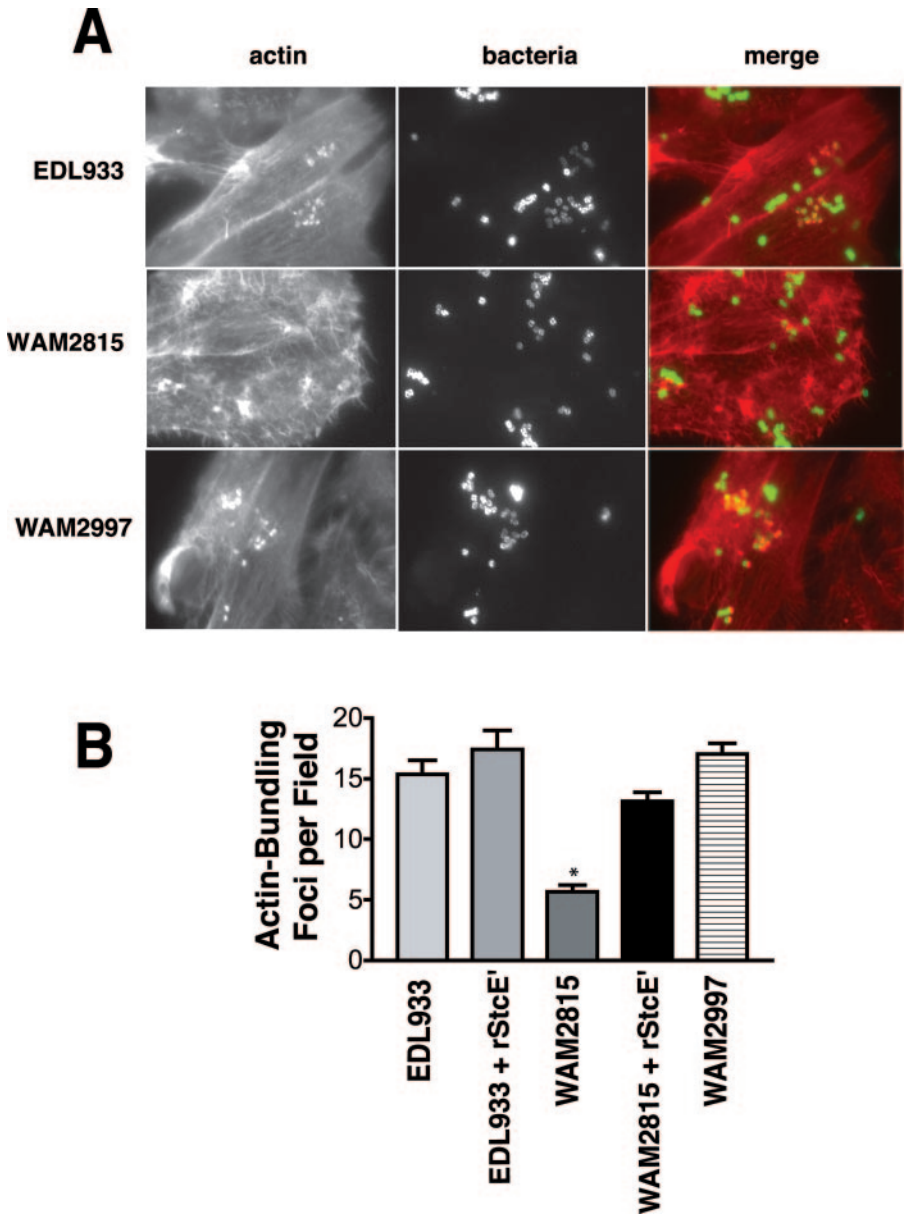

FIG. 3. Immunofluorescent images of HEp-2 cells infected by strains of *E. coli* O157:H7. HEp-2 cells were infected for 6 h with strains EDL933, WAM2815, and WAM2997, either with or without 2 μg of StcE. (A) Samples were stained for actin (red) and bacteria (green). Images are representative of data shown in panel B. (B) Actin-bundling foci were enumerated in sample fields for each infection. EDL933, wild-type EHEC; WAM2815, *stcE:cat*; WAM2997, WAM2815 complemented with the *stcE* gene. Data shown are the means and standard errors of the means of the combined results from five independent experiments. *, only the mean of sample WAM2815 was significantly different from the mean of the control, EDL933, based on the Dunnett posttest ($P < 0.001$).

StcE to the different substrates (e.g., C1-INH versus mucins) are different. These studies are currently under way in our lab.

Both saliva and intestinal mucus are composed of heavily glycosylated proteins, including the proteins of the mucin family. The structure of the mucins is characterized by a central region composed of tandem repeats that are serine/threonine rich and heavily glycosylated (55). The result is a protein with a "bottle-brush" conformation that can be 50 to 200 nm long and have molecular masses of hundreds to thousands of kilodaltons (68). Mucins can be cell bound or secreted and provide lubrication in the gastrointestinal tract. A firm, tightly adherent mucus layer over mucosal cells is 100 μm thick, while the loosely adherent mucus layer can be several times thicker (18, 66). Secreted mucins are gel forming, in part because they can form chains of disulfide-linked monomers. They also function to protect the single cell layer of the intestinal epithelium from the "outside world" of food and microorganisms constantly flowing through the intestine. The mucus layer is impermeable to large molecules but permeable to ions, sugars, nutrients, and other small molecules (68).

The emerging picture of StcE protein substrates is that they are heavily glycosylated, mostly via O-linkages, and that these

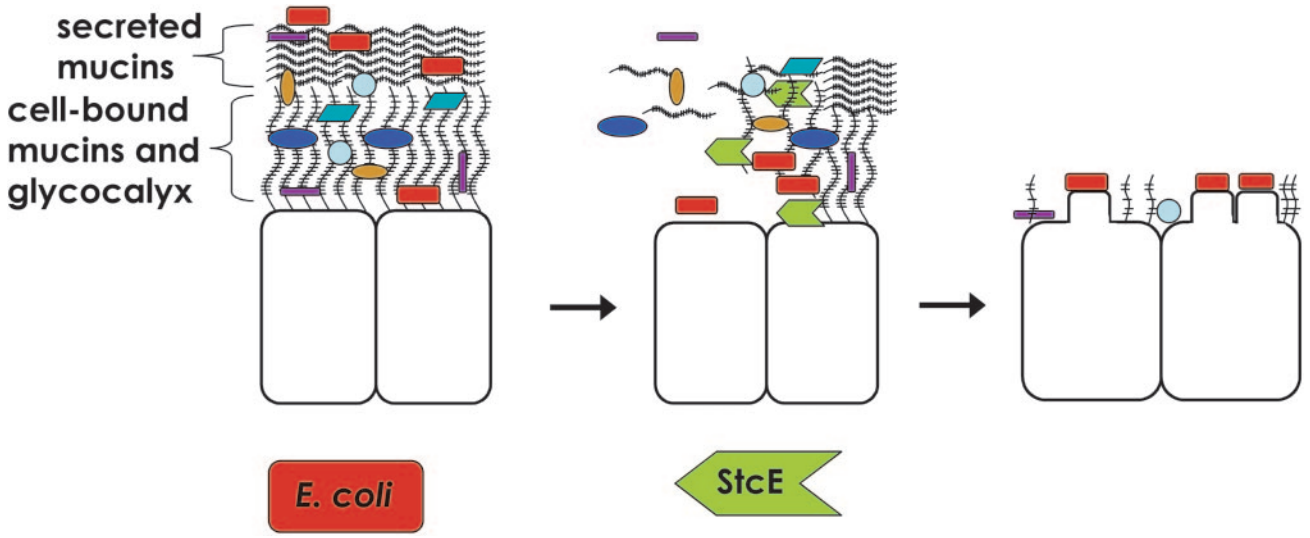

FIG. 4. Model for contribution of StcE to intimate adherence of *E. coli* O157:H7 to host cells. The StcE protease cleaves heavily glycosylated proteins of the glycocalyx and mucin layer, thereby reducing levels of competing organisms in the local environment and allowing the pathogen to come into close proximity with the host cell membrane. Red rectangles represent EHEC and green polygons represent StcE, while the other shapes and colors represent other microorganisms.

regions contain repeated amino acid sequences. The previously identified StcE substrate, C1-INH, shares these features with MUC7 and gp340. C1-INH has a highly conserved C-terminal serpin domain, but in contrast to other serpins, it has a unique 120-amino-acid N terminus that is heavily glycosylated. One region of the N terminus (residues 67 to 97) contains a series of imperfect repeats of the sequence Glx-Pro-Thr-Thr (11, 19). Recent results in our laboratory indicate that the C1-INH cleavage site for StcE lies between the N terminus and serpin domain, probably just C terminal to the glycosylated region and repeats (43). This suggests that other StcE substrates are likely to also contain repeated sequences and multiple O-glycosylations. It should be noted, however, that StcE is not a general mucinase because it does not cleave all glycosylated proteins, as evidenced by our inability to detect cleavage of gelatin (45), porcine gastric mucin, bovine submaxillary mucin, bovine fetuin, lactoferrin, or ovalbumin (data not shown).

Other enteropathogens produce proteins with mucinase activity. For instance, the HA/P zinc metalloprotease of *Vibrio cholerae* and the Pic protease of *Shigella flexneri* and enteroaggregative *E. coli* have both been shown to have mucinase activity. In both cases, however, the proteases are active against a wide variety of proteins. HA/P degrades fibronectin, ovomucin, and lactoferrin and is 61% identical to *Pseudomonas aeruginosa* elastase, another broad-spectrum protease (27, 32). In infections, HA/P has been suggested to be a factor mediating adherence as well as detachment (4, 5, 26). Pic is an autotransported serine protease that cleaves mucins from murine and bovine sources, including gelatin (33). Thus, in the class of enteropathogen mucinases, StcE seems unique in that it seems to have a more limited set of substrates.

MUC7 (also called MG2), one of the StcE substrates identified here, is found at high levels in saliva (100 to 980 μg/ml) as well as in lung secretions and tears. It has six repeats of 23 residues, 9 of which are possible glycosylation sites (10, 67). It binds a variety of bacteria, complexes with soluble immunoglobulin A and lactoferrin, and has antifungal activity (47, 63, 64). Roughly 70% of its molecular weight is composed of posttranslational modifications in the form of glycosylations (62).

The other substrate identified here, gp340, is one of at least two proteins resulting from alternative splicing of transcripts from the *DMBT1* gene (34, 53, 65). Known *DMBT1* proteins include DMBT1 and gp340 (previously identified as salivary agglutinin). They are members of the scavenger receptor cysteine-rich (SRCR) family and are primarily composed of 14 nearly identical SRCR domains. These are separated by SRCR-interspersed domains, which contain many potential O-glycosylation sites. On either side of the 13th SRCR domain lie two CUB (C1r/C1s Uegf Bmp1) domains. After the 14th SRCR domain is a ZP (zona pellucida) domain (9, 54). Each of these domain types is thought to mediate protein-protein interactions. gp340/DMBT1 have characteristics of a pattern-recognition receptor and bind the collectins SP-D and SP-A, which are also known to aggregate bacteria and are involved in mucosal inflammation responses (35, 65, 69). A peptide derived from one of the gp340/DMBT1 SRCR domains binds a wide variety of bacteria, including *E. coli* (9). This activity is thought to mediate clearance of pathogens from mucosal environments through creation of protein-bacteria aggregates and interactions with phagocytic cells (38).

Together, MUC7 and gp340 have been described to have important roles in innate oral defense mechanisms. Although the bacterium may not reside in the oral cavity for long periods of time, mastication mixes food with saliva proteins, which are all swallowed together and interact during initial digestion. MUC7 and gp340, as well as unknown StcE substrates, may be present throughout the mucus layers of the gastrointestinal tract.

Both mass spectrometry and immunoblotting with antiserum against MUC7 and a monoclonal antibody against gp340 failed to identify any proteins in samples of untreated or StcE-treated

HEp-2 cells (data not shown). Their identity may not be particularly important in terms of *E. coli* O157:H7 pathogenesis, given that HEp-2 cells are cervical cells (16, 57, 58). However, HEp-2 cells are the predominant model for studies of A/E formation, and results from this model system have been correlated with in vivo results (8, 24, 50). Interestingly, *DMBT1* gene products are produced and secreted by cells in the colon (52, 53). Therefore, in vivo, *DMBT1* gene products are likely to be colonic mucosal proteins that StcE acts upon to promote *E. coli* O157:H7 colonization and disease. In addition, *DMBT1* gene products are likely to have roles in innate defense in the colon similar to their role in the oral cavity. The cleavage of *DMBT1* gene products may also interfere with normal host signaling or cell differentiation, since the gene encodes a variety of domains as discussed above.

Previous studies have demonstrated that the common *E. coli* O157:H7 virulence plasmid, pO157, and therefore *stcE*, is not required for intimate adherence (30, 39, 70). Therefore, it is not surprising that the role of StcE in enhancing this activity has gone unrecognized. Many studies investigating the details of intimate adherence use enteropathogenic *E. coli* (EPEC) rather than EHEC strains. In vitro, EHEC does not adhere intimately as readily as EPEC (22). Growth under conditions unfavorable to *stcE* expression may partially explain this difference.

Our laboratory has recently shown that only a subset of EPEC strains contain the *stcE* gene, and the strain typically used in A/E studies, E2348/69, does not have *stcE* (44). EPEC disease is usually considered an event in the small intestine (56), while EHEC infects the large intestine. These two environments differ in many respects. The large intestine contains different mucins and 2- to 3-log-higher levels of normal flora than the small intestine (7, 18). The propensity to colonize different sites could be due to a number of factors influenced by characteristics of the host, the intestinal flora, and bacterial strain. For example, an EHEC strain that expressed an EPEC-derived intimin gene allowed EHEC to colonize tissue from a human small intestine (28).

A recent report suggested that the three main EPEC proteins involved in general adherence to differentiated Caco-2 cells were BFP, EspA, and intimin (17). BFP mediates rapid adherence (within 10 min), EspA is responsible for a low level of adherence under similar conditions, and intimin imparts no adherence unless the host cells have been preinfected with a Tir-producing strain. Among these, BFP seems to be most important for adherence. Indeed, strains with inactivated BFP are deficient for microcolony formation, show reduced adherence to host cells, and cause less diarrhea in volunteers (8, 17, 31). However, there is a class of "atypical" EPEC strains that lack the plasmid that encodes BFP and cause disease in adults in industrialized countries, as opposed to infants in underdeveloped countries, which is the typical population affected by EPEC (2, 25, 60, 71). Interestingly, the *stcE*-positive EPEC strains seem to be restricted to a group of atypical EPEC of the O55:H7 serotype (44). Since some atypical EPEC strains lack BFP but have *stcE*, it is possible that StcE helps compensate for the deficiency of BFP-mediated adherence, possibly by increasing the efficiency of EspA-mediated adherence. Interestingly, Mack et al. recently reported that addition of mucin-enriched medium can inhibit adherence of EPEC strain E2348/69 to HEp-2 cells (48).

Our multifunctional model of StcE activity has precedents in EHEC pathogenesis. For example, the Tir protein is an EHEC receptor as well as a cytoplasmic effector that participates in host cell signaling and actin organization (15, 29). The EspA-composed filaments of the T3SS serve as adhesins as well as channels for protein transport from bacterium to host cell (1, 17, 42, 59). Characterization of the functional consequences of StcE activity on its substrates and further identification of additional substrates will lead to a better understanding of its role in *E. coli* O157:H7 pathogenesis. Furthermore, the association of *stcE* with an atypical EPEC lineage leads to a potential use of *stcE* as an epidemiological and evolutionary marker to describe atypical EPEC strains, which are an important emerging class of pathogenic *E. coli* (2, 25, 60, 71).

ACKNOWLEDGMENTS

This work was supported by NIH grant ROI AI051735. T.E.G. received support from the Biotechnology Training Program funded by NIH grant 5T32GM08349.

We thank J. Bangs for use of his microscope, H. Yu and E. Turner of the UW Chemistry Department for helpful discussions on viscosity, and J. G. M. Bolscher for the kind gift of MUC7 antiserum. We also thank A. Atterbury, C. Cowles, K. Nicol, and P. Roesch for helpful discussions about the project and manuscript.

REFERENCES

1. **Abe, A., U. Heczko, R. G. Hegele, and B. Brett Finlay.** 1998. Two enteropathogenic *Escherichia coli* type III secreted proteins, EspA and EspB, are virulence factors. J. Exp. Med. **188:**1907–1916.
2. **Afset, J. E., K. Bergh, and L. Bevanger.** 2003. High prevalence of atypical enteropathogenic *Escherichia coli* (EPEC) in Norwegian children with diarrhoea. J. Med. Microbiol. **52:**1015–1019.
3. **Arnold, K. W., and C. W. Kaspar.** 1995. Starvation- and stationary-phase-induced acid tolerance in *Escherichia coli* O157:H7. Appl. Environ. Microbiol. **61:**2037–2039.
4. **Benitez, J. A., A. J. Silva, and R. A. Finkelstein.** 2001. Environmental signals controlling production of hemagglutinin/protease in *Vibrio cholerae*. Infect. Immun. **69:**6549–6553.
5. **Benitez, J. A., R. G. Spelbrink, A. Silva, T. E. Phillips, C. M. Stanley, M. Boesman-Finkelstein, and R. A. Finkelstein.** 1997. Adherence of *Vibrio cholerae* to cultured differentiated human intestinal cells: an in vitro colonization model. Infect. Immun. **65:**3474–3477.
6. **Benjamin, M. M., and A. R. Datta.** 1995. Acid tolerance of enterohemorrhagic *Escherichia coli.* Appl. Environ. Microbiol. **61:**1669–1672.
7. **Berg, R. D.** 1996. The indigenous gastrointestinal microflora. Trends Microbiol. **4:**430–435.
8. **Bieber, D., S. W. Ramer, C. Y. Wu, W. J. Murray, T. Tobe, R. Fernandez, and G. K. Schoolnik.** 1998. Type IV pili, transient bacterial aggregates, and virulence of enteropathogenic *Escherichia coli.* Science **280:**2114–2118.
9. **Bikker, F. J., A. J. Ligtenberg, K. Nazmi, E. C. Veerman, W. van't Hof, J. G. Bolscher, A. Poustka, A. V. Nieuw Amerongen, and J. Mollenhauer.** 2002. Identification of the bacteria-binding peptide domain on salivary agglutinin (gp340/DMBT1), a member of the scavenger receptor cysteine-rich superfamily. J. Biol. Chem. **277:**32109–32115.
10. **Bobek, L. A., H. Tsai, A. R. Biesbrock, and M. J. Levine.** 1993. Molecular cloning, sequence, and specificity of expression of the gene encoding the low molecular weight human salivary mucin (MUC7). J. Biol. Chem. **268:**20563–20569.
11. **Bock, S. C., K. Skriver, E. Nielsen, H. C. Thogersen, B. Wiman, V. H. Donaldson, R. L. Eddy, J. Marrinan, E. Radziejewska, R. Huber, et al.** 1986. Human C1 inhibitor: primary structure, cDNA cloning, and chromosomal localization. Biochemistry **25:**4292–4301.
12. **Brunder, W., H. Schmidt, and H. Karch.** 1996. KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. Microbiology **142**(Pt. 11)**:**3305–3315.
13. **Burland, V., Y. Shao, N. T. Perna, G. Plunkett, H. J. Sofia, and F. R. Blattner.** 1998. The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. Nucleic Acids Res. **26:**4196–4204.
14. **Caliezi, C., W. A. Wuillemin, S. Zeerleder, M. Redondo, B. Eisele, and C. E. Hack.** 2000. C1-esterase inhibitor: an anti-inflammatory agent and its poten-

tial use in the treatment of diseases other than hereditary angioedema. Pharmacol. Rev. **52:**91–112.
15. **Campellone, K. G., and J. M. Leong.** 2003. Tails of two Tirs: actin pedestal formation by enteropathogenic *E. coli* and enterohemorrhagic *E. coli* O157:H7. Curr. Opin. Microbiol. **6:**82–90.
16. **Chen, T. R.** 1988. Re-evaluation of HeLa, HeLa S3, and HEp-2 karyotypes. Cytogenet. Cell Genet. **48:**19–24.
17. **Cleary, J., L. C. Lai, R. K. Shaw, A. Straatman-Iwanowska, M. S. Donnenberg, G. Frankel, and S. Knutton.** 2004. Enteropathogenic *Escherichia coli* (EPEC) adhesion to intestinal epithelial cells: role of bundle-forming pili (BFP), EspA filaments and intimin. Microbiology **150:**527–538.
18. **Corfield, A. P., D. Carroll, N. Myerscough, and C. S. Probert.** 2001. Mucins in the gastrointestinal tract in health and disease. Front. Biosci. **6:**D1321–D1357.
19. **Coutinho, M., K. S. Aulak, and A. E. Davis.** 1994. Functional analysis of the serpin domain of C1 inhibitor. J. Immunol. **153:**3648–3654.
20. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
21. **DeLoney, C. R., T. M. Bartley, and K. L. Visick.** 2002. Role for phosphoglucomutase in *Vibrio fischeri-Euprymna scolopes* symbiosis. J. Bacteriol. **184:**5121–5129.
22. **DeVinney, R., M. Stein, D. Reinscheid, A. Abe, S. Ruschkowski, and B. B. Finlay.** 1999. Enterohemorrhagic *Escherichia coli* O157:H7 produces Tir, which is translocated to the host cell membrane but is not tyrosine phosphorylated. Infect. Immun. **67:**2389–2398.
23. **Donnenberg, M. S., and J. P. Nataro.** 1995. Methods for studying adhesion of diarrheagenic *Escherichia coli.* Methods Enzymol. **253:**324–336.
24. **Donnenberg, M. S., S. Tzipori, M. L. McKee, A. D. O'Brien, J. Alroy, and J. B. Kaper.** 1993. The role of the *eae* gene of enterohemorrhagic *Escherichia coli* in intimate attachment in vitro and in a porcine model. J. Clin. Investig. **92:**1418–1424.
25. **Dulguer, M. V., S. H. Fabbricotti, S. Y. Bando, C. A. Moreira-Filho, U. Fagundes-Neto, and I. C. Scaletsky.** 2003. Atypical enteropathogenic *Escherichia coli* strains: phenotypic and genetic profiling reveals a strong association between enteroaggregative *E. coli* heat-stable enterotoxin and diarrhea. J. Infect. Dis. **188:**1685–1694.
26. **Finkelstein, R. A., M. Boesman-Finkelstein, Y. Chang, and C. C. Hase.** 1992. *Vibrio cholerae* hemagglutinin/protease, colonial variation, virulence, and detachment. Infect. Immun. **60:**472–478.
27. **Finkelstein, R. A., M. Boesman-Finkelstein, and P. Holt.** 1983. Vibrio cholerae hemagglutinin/lectin/protease hydrolyzes fibronectin and ovomucin: F. M. Burnet revisited. Proc. Natl. Acad. Sci. USA **80:**1092–1095.
28. **Fitzhenry, R. J., D. J. Pickard, E. L. Hartland, S. Reece, G. Dougan, A. D. Phillips, and G. Frankel.** 2002. Intimin type influences the site of human intestinal mucosal colonisation by enterohaemorrhagic *Escherichia coli* O157:H7. Gut **50:**180–185.
29. **Frankel, G., A. D. Phillips, I. Rosenshine, G. Dougan, J. B. Kaper, and S. Knutton.** 1998. Enteropathogenic and enterohaemorrhagic *Escherichia coli*: more subversive elements. Mol. Microbiol. **30:**911–921.
30. **Fratamico, P. M., S. Bhaduri, and R. L. Buchanan.** 1993. Studies on *Escherichia coli* serotype O157:H7 strains containing a 60-MDa plasmid and on 60-MDa plasmid-cured derivatives. J. Med. Microbiol. **39:**371–381.
31. **Giron, J. A., A. S. Ho, and G. K. Schoolnik.** 1991. An inducible bundle-forming pilus of enteropathogenic *Escherichia coli.* Science **254:**710–713.
32. **Hase, C. C., and R. A. Finkelstein.** 1991. Cloning and nucleotide sequence of the *Vibrio cholerae* hemagglutinin/protease (HA/protease) gene and construction of an HA/protease-negative strain. J. Bacteriol. **173:**3311–3317.
33. **Henderson, I. R., J. Czeczulin, C. Eslava, F. Noriega, and J. P. Nataro.** 1999. Characterization of Pic, a secreted protease of *Shigella flexneri* and enteroaggregative *Escherichia coli*. Infect. Immun. **67:**5587–5596.
34. **Holmskov, U., P. Lawson, B. Teisner, I. Tornoe, A. C. Willis, C. Morgan, C. Koch, and K. B. Reid.** 1997. Isolation and characterization of a new member of the scavenger receptor superfamily, glycoprotein-340 (gp340), as a lung surfactant protein-D binding molecule. J. Biol. Chem. **272:**13743–13749.
35. **Holmskov, U., J. Mollenhauer, J. Madsen, L. Vitved, J. Gronlund, I. Tornoe, A. Kliem, K. B. Reid, A. Poustka, and K. Skjodt.** 1999. Cloning of gp340, a putative opsonin receptor for lung surfactant protein D. Proc. Natl. Acad. Sci. USA **96:**10794–10799.
36. **Jerse, A. E., and J. B. Kaper.** 1991. The *eae* gene of enteropathogenic *Escherichia coli* encodes a 94-kilodalton membrane protein, the expression of which is influenced by the EAF plasmid. Infect. Immun. **59:**4302–4309.
37. **Jerse, A. E., J. Yu, B. D. Tall, and J. B. Kaper.** 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc. Natl. Acad. Sci. USA **87:**7839–7843.
38. **Kang, W., and K. B. Reid.** 2003. DMBT1, a regulator of mucosal homeostasis through the linking of mucosal defense and regeneration? FEBS Lett. **540:**21–25.
39. **Karch, H., J. Heesemann, R. Laufs, A. D. O'Brien, C. O. Tacket, and M. M. Levine.** 1987. A plasmid enterohemorrhagic *Escherichia coli* O157:H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. Infect. Immun. **55:**455–461.
40. **Kenny, B., R. DeVinney, M. Stein, D. J. Reinscheid, E. A. Frey, and B. B. Finlay.** 1997. Enteropathogenic E. coli (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell **91:**511–520.
41. **Knutton, S., T. Baldwin, P. H. Williams, and A. S. McNeish.** 1989. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli.* Infect. Immun. **57:**1290–1298.
42. **Knutton, S., I. Rosenshine, M. J. Pallen, I. Nisan, B. C. Neves, C. Bain, C. Wolff, G. Dougan, and G. Frankel.** 1998. A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells. EMBO J. **17:**2166–2176.
43. **Lathem, W. W., T. Bergsbaken, and R. A. Welch.** 2004. Potentiation of C1 esterase inhibitor by StcE, a metalloprotease secreted by *Escherichia coli* O157:H7. J. Exp. Med. **199:**1077–1087.
44. **Lathem, W. W., T. Bergsbaken, S. E. Witowski, N. T. Perna, and R. A. Welch.** 2002. Acquisition of StcE, a C1 esterase inhibitor-specific metalloprotease, by *Escherichia coli* O157:H7. J. Infect. Dis. **187:**1907–1914.
45. **Lathem, W. W., T. E. Grys, S. E. Witowski, A. G. Torres, J. B. Kaper, P. I. Tarr, and R. A. Welch.** 2002. StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. Mol. Microbiol. **45:**277–288.
46. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1:**190–206.
47. **Liu, B., S. A. Rayment, C. Gyurko, F. G. Oppenheim, G. D. Offner, and R. F. Troxler.** 2000. The recombinant N-terminal region of human salivary mucin MG2 (MUC7) contains a binding domain for oral streptococci and exhibits candidacidal activity. Biochem. J. **345**(Pt. 3)**:**557–564.
48. **Mack, D. R., S. Michail, S. Wei, L. McDougall, and M. A. Hollingsworth.** 1999. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. Am. J. Physiol. **276:**G941–G950.
49. **McDaniel, T. K., K. G. Jarvis, M. S. Donnenberg, and J. B. Kaper.** 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proc. Natl. Acad. Sci. USA **92:**1664–1668.
50. **McKee, M. L., A. R. Melton-Celsa, R. A. Moxley, D. H. Francis, and A. D. O'Brien.** 1995. Enterohemorrhagic *Escherichia coli* O157:H7 requires intimin to colonize the gnotobiotic pig intestine and to adhere to HEp-2 cells. Infect. Immun. **63:**3739–3744.
51. **Mellies, J. L., S. J. Elliott, V. Sperandio, M. S. Donnenberg, and J. B. Kaper.** 1999. The Per regulon of enteropathogenic Escherichia coli: identification of a regulatory cascade and a novel transcriptional activator, the locus of enterocyte effacement (LEE)-encoded regulator (Ler). Mol. Microbiol. **33:**296–306.
52. **Mollenhauer, J., S. Herbertz, B. Helmke, G. Kollender, I. Krebs, J. Madsen, U. Holmskov, K. Sorger, L. Schmitt, S. Wiemann, H. F. Otto, H. J. Grone, and A. Poustka.** 2001. Deleted in malignant brain tumors 1 is a versatile mucin-like molecule likely to play a differential role in digestive tract cancer. Cancer Res. **61:**8880–8886.
53. **Mollenhauer, J., S. Herbertz, U. Holmskov, M. Tolnay, I. Krebs, A. Merlo, H. D. Schroder, D. Maier, F. Breitling, S. Wiemann, H. J. Grone, and A. Poustka.** 2000. *DMBT1* encodes a protein involved in the immune defense and in epithelial differentiation and is highly unstable in cancer. Cancer Res. **60:**1704–1710.
54. **Mollenhauer, J., U. Holmskov, S. Wiemann, I. Krebs, S. Herbertz, J. Madsen, P. Kioschis, J. F. Coy, and A. Poustka.** 1999. The genomic structure of the *DMBT1* gene: evidence for a region with susceptibility to genomic instability. Oncogene **18:**6233–6240.
55. **Moniaux, N., F. Escande, N. Porchet, J. P. Aubert, and S. K. Batra.** 2001. Structural organization and classification of the human mucin genes. Front. Biosci. **6:**D1192–D1206.
56. **Nataro, J. P., and J. B. Kaper.** 1998. Diarrheagenic *Escherichia coli.* Clin. Microbiol. Rev. **11:**142–201.
57. **Nelson-Rees, W. A., L. Hunter, G. J. Darlington, and S. J. O'Brien.** 1980. Characteristics of HeLa strains: permanent vs. variable features. Cytogenet. Cell. Genet. **27:**216–231.
58. **Nelson-Rees, W. A., V. M. Zhdanov, P. K. Hawthorne, and R. R. Flandermeyer.** 1974. HeLa-like marker chromosomes and type-A variant glucose-6-phosphate dehydrogenase isoenzyme in human cell cultures producing Mason-Pfizer monkey virus-like particles. J. Natl. Cancer Inst. **53:**751–757.
59. **Neves, B. C., R. K. Shaw, G. Frankel, and S. Knutton.** 2003. Polymorphisms within EspA filaments of enteropathogenic and enterohemorrhagic *Escherichia coli.* Infect. Immun. **71:**2262–2265.
60. **Pelayo, J. S., I. C. Scaletsky, M. Z. Pedroso, V. Sperandio, J. A. Giron, G. Frankel, and L. R. Trabulsi.** 1999. Virulence properties of atypical EPEC strains. J. Med. Microbiol. **48:**41–49.
61. **Perna, N. T., G. Plunkett III, V. Burland, B. Mau, J. D. Glasner, D. J. Rose, G. F. Mayhew, P. S. Evans, J. Gregor, H. A. Kirkpatrick, G. Postai, J. Hackett, S. Klink, A. Boutin, Y. Shao, L. Miller, E. J. Grotbeck, N. W. Davis, A. Lim, E. T. Dimalanta, K. D. Potamousis, J. Apodaca, T. S. Anantharaman, J. Lin, G. Yen, D. C. Schwartz, R. A. Welch, and F. R. Blattner.** 2001.

Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature **409:**529–533.

62. **Prakobphol, A., M. J. Levine, L. A. Tabak, and M. S. Reddy.** 1982. Purification of a low-molecular-weight, mucin-type glycoprotein from human submandibular-sublingual saliva. Carbohydr. Res. **108:**111–122.
63. **Prakobphol, A., K. Tangemann, S. D. Rosen, C. I. Hoover, H. Leffler, and S. J. Fisher.** 1999. Separate oligosaccharide determinants mediate interactions of the low-molecular-weight salivary mucin with neutrophils and bacteria. Biochemistry **38:**6817–6825.
64. **Prakobphol, A., K. A. Thomsson, G. C. Hansson, S. D. Rosen, M. S. Singer, N. J. Phillips, K. F. Medzihradszky, A. L. Burlingame, H. Leffler, and S. J. Fisher.** 1998. Human low-molecular-weight salivary mucin expresses the sialyl lewis-X determinant and has L-selectin ligand activity. Biochemistry **37:**4916–4927.
65. **Prakobphol, A., F. Xu, V. M. Hoang, T. Larsson, J. Bergstrom, I. Johansson, L. Frangsmyr, U. Holmskov, H. Leffler, C. Nilsson, T. Boren, J. R. Wright, N. Stromberg, and S. J. Fisher.** 2000. Salivary agglutinin, which binds *Streptococcus mutans* and *Helicobacter pylori*, is the lung scavenger receptor cysteine-rich protein gp340. J. Biol. Chem. **275:**39860–39866.
66. **Pullan, R. D., G. A. Thomas, M. Rhodes, R. G. Newcombe, G. T. Williams, A. Allen, and J. Rhodes.** 1994. Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. Gut **35:**353–359.
67. **Seregni, E., C. Botti, S. Massaron, C. Lombardo, A. Capobianco, A. Bogni, and E. Bombardieri.** 1997. Structure, function and gene expression of epithelial mucins. Tumori **83:**625–632.
68. **Strous, G. J., and J. Dekker.** 1992. Mucin-type glycoproteins. Crit. Rev. Biochem. Mol. Biol. **27:**57–92.
69. **Tino, M. J., and J. R. Wright.** 1999. Glycoprotein-340 binds surfactant protein-A (SP-A) and stimulates alveolar macrophage migration in an SP-A-independent manner. Am. J. Respir. Cell. Mol. Biol. **20:**759–768.
70. **Toth, I., M. L. Cohen, H. S. Rumschlag, L. W. Riley, E. H. White, J. H. Carr, W. W. Bond, and I. K. Wachsmuth.** 1990. Influence of the 60-megadalton plasmid on adherence of *Escherichia coli* O157:H7 and genetic derivatives. Infect. Immun. **58:**1223–1231.
71. **Trabulsi, L. R., R. Keller, and T. A. Tardelli Gomes.** 2002. Typical and atypical enteropathogenic *Escherichia coli.* Emerg. Infect. Dis. **8:**508–513.
72. **Veerman, E. C., J. G. Bolscher, B. J. Appelmelk, E. Bloemena, T. K. van den Berg, and A. V. Nieuw Amerongen.** 1997. A monoclonal antibody directed against high M_r salivary mucins recognizes the SO3-3Gal β1-3GlcNAc moiety of sulfo-Lewis(a): a histochemical survey of human and rat tissue. Glycobiology **7:**37–43.

Editor: A. D. O'Brien